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## EVOKING CONDITIONED FEAR BY ELECTRICAL STIMULATION OF SUBCORTICAL STRUCTURES IN MONKEY

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The work of Hess (9, 10), Ranson and Magoun (8), Kaada (11), MacLean and Delgado (12), Gastaut and collaborators (8), and Masserman (14) has demonstrated that the neocortex, diencephalon, and rhinencephalon play important roles in the integration of emotional reactions. Electrical stimulation of all these subcortical structures has elicited a wide variety of the behavioral components usually associated with fear, rage, and anger. Masserman (14), however, was unable to condition an escape response to hypothalamic stimulation, or even to elicit an escape response by such stimulation in animals which had already been conditioned to escape to auditory stimuli. He concluded, therefore, much as Bard had done earlier in connection with lesions placed in the hypothalamus, that "these pseudo-affective responses differ from those in integrated emotional states in that the hypothalamic reactions are not adapted to external circumstances" (1, p. 633). Delgado (6), on the other hand, observed that animals in which emotional responses were being evoked by stimulating in subcortical structures other than the hypothalamus soon began to exhibit anxiety-like responses when placed in the apparatus prior to stimulation. These observations were incidental to the main purpose of that study, and the phenomenon may have been an artifact of certain uncontrolled variables. The present study was designed to determine whether electrical stimulation in these same subcortical structures would evoke a fear response which had been conditioned to an auditory stimulus. Positive results would suggest that the emotional behavior mediated by these subcortical struc-

tures, unlike that mediated by the hypothalamus, can be adapted to external circumstances.

### METHOD

#### Subjects

Ten monkeys (*Macaca mulatta*), ranging in weight from 2.0 kg. to 3.0 kg., were housed in individual cages and maintained on a standard synthetic diet calculated to provide 80 cal/kg of body weight daily. One-half orange three times a week supplemented the diet. Upon completion of preoperative training, an assembly of six needle electrodes was permanently implanted within each hemisphere at the positions in the rhinencephalon, diencephalon, and mesencephalon from which Delgado (6) had previously elicited emotional behavior.

#### Apparatus

##### Needle Electrodes

Enameled or Teflon-coated stainless steel wire, 0.005 in. in diameter, was straightened by stretching and cut into six graduated lengths: the first 10 cm. long, and each succeeding piece 4 mm. shorter. Each wire was scraped bare of insulation for 1 mm. at the tip, and for about 5 mm. at the socket end. Six such wires were then cemented together side by side with Plexiglas dissolved in dichlorethylene, each bare tip 4 mm. from the next. The socket ends were soldered to a seven-pin miniature radio tube socket in an identifiable order. A bare stainless steel wire was soldered to the middle pin to serve as the indifferent lead. These seven wires were insulated from one another by flooding the soldered ends with INSLX-E33 clear. This assembly provided a six-point multilead electrode by means of which the brain could be stimulated at six vertical levels.

##### Electrical Stimulator

The two-channel Hols-Delgado stimulator providing bidirectional rectangular pulses was connected to the electrode by means of a light-weight, flexible multilead cable. Frequency was kept constant at 100 cycles per second, and pulse duration at .35 msec.; amplitude was varied from 1 to 10 v. Monitoring the current with a dual-beam oscilloscope (DuMont Type 322) indicated that current of 0.25 to 4 ma. passed through the brain when 1 to 10 v. was applied. Stimulation was unilateral and monopolar. In some cases bipolar stimulation between adjacent points was also used to check the results of monopolar stimulation. In each stimulation trial the animals were continuously stimulated until they responded or until 40 sec. had elapsed.

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### Testing Apparatus

The animal was carried to an air-conditioned, sound-proofed, darkened testing room, where it was released into the testing cage. On stimulation days before being released into the testing cage the multilead cable was plugged into the electrode socket. The animal was free to move about in the testing cage even when the multilead cable was plugged in. The testing cage was a Wisconsin General Test Apparatus (WGTA) modified by Waterhouse (20) to contain an electric grid floor through which shock could be administered to the animal's feet as described by Beck, Runyon, and Waterhouse (2). The level of shock was regulated so that it produced apparent signs of discomfort in the monkey without producing tetany. Lifting the one-way vision screen started a timer and, either immediately or after 2 sec., electrified the grid. Lifting the screen also initiated either the high tone, the low tone, the no-tone, or the electrical stimulation. In addition, lifting the screen exposed to the animal two cups placed 12 in. apart, each fastened to a separate side. Turning over a cup stopped the timer, the current in the grid, and the tones or the electrical stimulation. The time elapsed between the raising of the screen and the turning of the cups was called the "response time." The monkeys were trained to pull in and overturn the left cup in response to the high tone to stop (escape) and later to avoid, the shock to the feet, and the right cup in response to the low tone to obtain a peanut reward. This training proceeded as described below.

### Procedure

#### Training

Twenty-five trials of each of the two responses were distributed at random over the day's 50 trials. The trials were separated by 5 to 10 sec. The training was continued until the animal was correct on all trials and avoided shock by anticipating every fear (high-tone) trial on five successive days. Then 10 trials preceded by no-tone were presented at random among the other 50 trials for a total of 60 trials. The no-tone trial consisted at first of confronting the monkey with various stimuli such as blinking lights, trainer's gloves, and banging noises as the screen was raised. Gradually these novel stimuli were omitted so that finally the no-tone condition consisted in simply raising the screen. Training continued under these conditions until on three successive days the animal responded to the left cup only for the conditioned fear stimulus, and to the right cup for everything but the conditioned fear stimulus, i.e., on both the low-tone and no-tone trials. The electrodes were then implanted. Training was resumed within a few days after operation and was continued until on three successive days the animals did not make an error either by going to the wrong cup or by failing to avoid shock.

#### Testing

*Selection of the stimulation intensity.* The animal was placed on the observation stage described by MacLean and Delgado (12), and all the leads of both electrodes were plugged into a flexible cable leading to the stimu-

lator. With frequency and pulse duration constant at 100 cps and .35 msec., respectively, the voltage was varied in 0.1-v. intervals from 1.0 to a maximum of 10.0 v., repeating each level three times in one ascending and one descending series for each lead. The *E* recorded whether or not he observed an effect and on the basis of earlier investigations categorized the behavior into fear responses, motor effects, or inhibition of movement. The lowest voltage at which an effect was apparent every time in both series (six out of six) was called the "effective threshold" for that lead. This was the intensity of the stimulation used for that lead in the systematic testing.

*Systematic testing.* Each day that the animal was placed in the Waterhouse apparatus only three leads were plugged in, one in one hemisphere and two in the other. The leads were selected so that (a) on any one day stimulation was applied through one lead from which fear responses had been elicited, one from which either motor effects or inhibition had been elicited, and one from which there had been no apparent effect. (b) The same lead was selected on three different days, separated by as many days as possible, usually three.

A day's session consisted of 60 trials—15 stimulation (5 through each of three leads), 20 low-tone, 20 high-tone (fear), and 5 no-tone trials—presented in a balanced order such that neither (a) two stimulation trials, nor (b) a fear trial and a stimulation trial expected to elicit a fear response, were juxtaposed. The systematic testing was completed in 12 testing days and provided observations on a total of 15 stimulation trials through each lead.

*Testing the limits.* Upon completion of the 12 days of systematic testing, several variations in procedure were introduced. In one of these, the low (food) tone, was sounded simultaneously with applying stimulation through a lead from which fear had been elicited. A conditioned fear response in the presence of this competing drive was considered a definitive demonstration of the capacity of the stimulation to evoke fear responses. In another of these variations, the high tone or low tone was sounded simultaneously with applying stimulation through a lead from which inhibition had been elicited. Failure to respond in the presence of these competing drives was considered a definitive demonstration of an inhibitory effect of stimulation. Toward the end of this testing period, stimulation was applied only through leads from which fear responses had been elicited. Thus, an animal received a total of approximately 40 trials in which the conditioned fear response was elicited by substituting electrical stimulation for the conditioned stimulus.

### Surgical Procedure

Surgical procedures, construction of electrodes, and the method of implantation have been reported by Delgado (3, 5) and Rosvold and Delgado (19). Briefly, the method was as follows: with the animal under anesthesia and placed in the Horsley-Clarke instrument, the assembly of needle electrodes was lowered through holes in the skull drilled at points designated by Olzewski (16) to locate the various intracerebral structures to be investigated. The electrodes were

secured in place by dental cement and steel wire ties through the skull. Electrodes thus implanted remained in place over many months and permitted daily testing of the unanaesthetized animal while it was being stimulated.

#### *Anatomical Procedure*

Following completion of the behavioral observations, the animals were sacrificed and the brains prepared for histological examination as described by Rosvold and Delgado (19). Cells were stained by the method of Nissl, fibers by the method of Spielmeier.<sup>2</sup> As a check, the Klüver method of staining cells and fibers simultaneously was used in some brains. Sections were examined to determine the anatomical location of the electrode leads. The sections in the plane of the electrode were photographed, and the enlarged prints were examined to identify the structures surrounding the leads. Each histological section was then magnified through a photographic enlarger and the image traced, marking the channel of the electrode and location of the leads. These findings, together with tissue changes, were verified microscopically. A final drawing of the section was prepared by comparing the tracing and the photograph.

### RESULTS

#### *Responses to the Conditioned Stimuli*

The interpolation of stimulation trials during the testing sessions did not alter significantly either the accuracy or the response times of the conditioned responses. Pulling in and overturning the left cup in response to the high tone (conditioned fear response, henceforth called CFR) continued to be a very energetic, fast response with a mean response time of 1.78 sec. Pulling in and overturning the right cup in response to the low tone (conditioned food response) and in response to the no-tone (also a conditioned food response—the rising screen served as the conditioned stimulus) continued to be identical and relatively slow responses, each with a mean response time of 4.24 sec.

#### *Responses to the Electrical Stimulation*

Stimulation trials were identical to the no-tone trial (i.e., the only external stimulus was the rising screen) except that electrical stimulation was applied through one of the leads. The effect of the stimulation was to elicit one of five responses: (a) A response identical to that which had been made to the high-tone. This response was indistinguishable from the CFR with respect to response time and accom-

panying autonomic signs. It was therefore called an "elicited fear response" (FEAR). (b) A response identical to that which had been made to the low-tone or no-tone. This response was indistinguishable from the conditioned food response with respect to response time and absence of autonomic signs. However, the interpretation of a response to the food cup during stimulation is equivocal; i.e., it could be interpreted as indicating that stimulation elicited a food response, or that stimulation had no effect, the response to the food cup being simply the CR to the rising screen in the absence of the high (fear) tone. Therefore, the more conservative interpretation was made, namely, that a response to the right cup indicated that the stimulation had "no apparent effect" (NAE). (c) A response to the food cup which was accompanied by pronounced "motor effects" (MOTOR). (d) A response to neither cup. The monkey sat motionless in the cage slowly turning its head from side to side for the 30 sec. during which the current was on, after which it moved abruptly to the food cup. Such a failure to respond while the current was on was called an "inhibited response" (INHIBIT). (e) A response in which the animal did not overturn either cup for 10 to 39 sec., but moved cautiously about the cage, frequently as though searching the floor. This response was called "a slow response" (SLOW).

Every time a stimulating current was passed through a particular lead, the same response was invariably elicited. Even in those trials which tested the limits, i.e., in which a CS was sounded during the stimulation, the type of response that resulted was related to the structure being stimulated rather than the CS, always being different from that to be expected in response to the CS.

#### *Anatomical Results*

The structures from which these responses were elicited are identified in Figure 1, which contains reproductions of the sections in the plane of the electrode tracts from seven different hemispheres. Reproductions of sections from the 13 other hemispheres (bilateral implantation in 10 animals, making 20 hemispheres) are not shown because they are similar to one or another of the seven. Thus, the

<sup>2</sup> Miss Beatrix Egli prepared the histological slides.

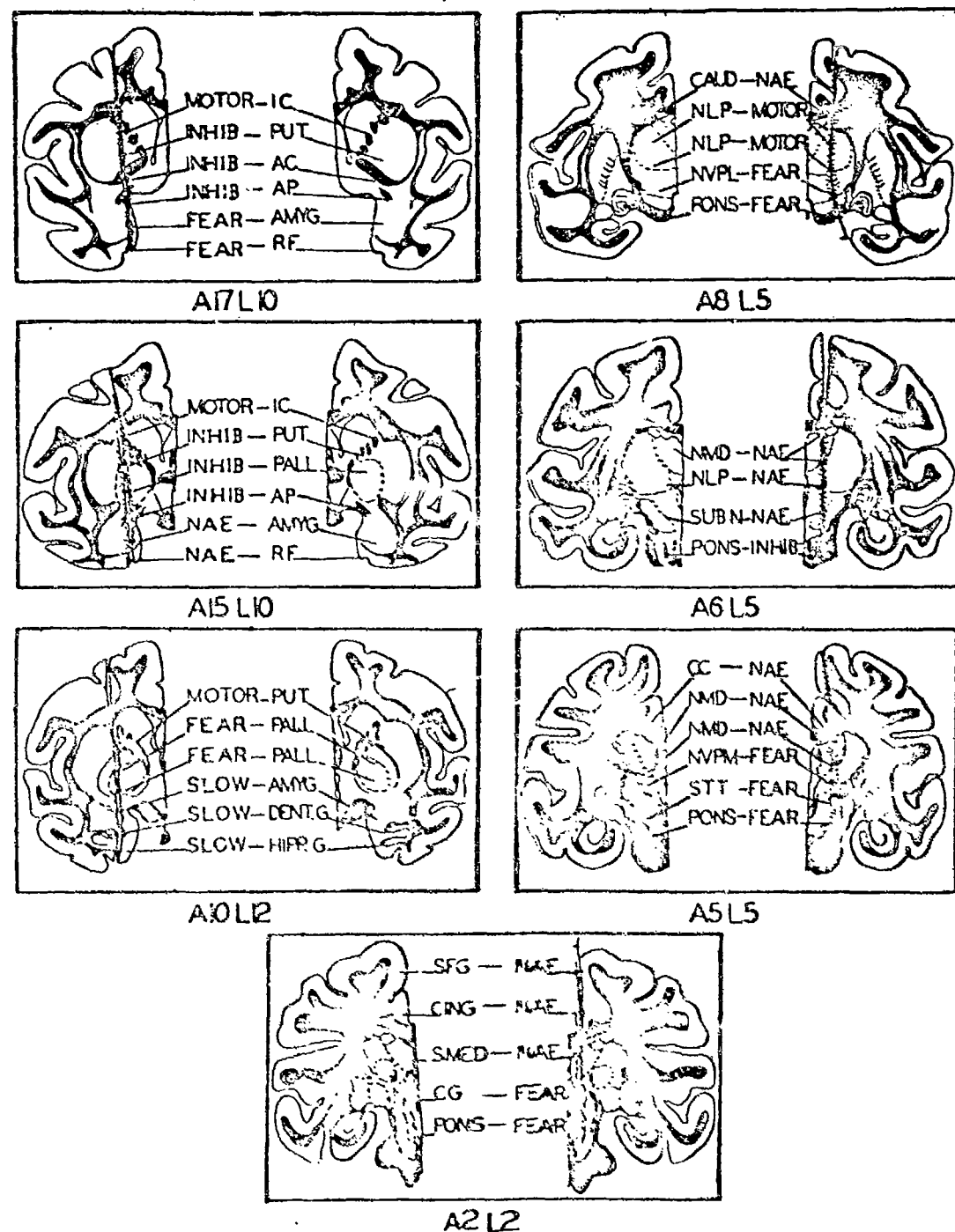


Fig. 1. Camera lucida tracings of sections in the plane of the electrode from at least one animal implanted at each of the Horsley-Clarke positions investigated in this study. A:17 indicates the anterior H-C coordinate of the electrode placement, L:10, the lateral. The black dots in the channel of the electrode represent the points of stimulation. Their positions within the brain appear somewhat distorted as a result of fixing and cutting processes. INHIB = inhibitory response; NAE = no apparent effect; IC = internal capsule, PUT = putamen, AC = anterior commissure, AP = ansa peduncularis, AMYG = amygdala, RF = rhinal fissure, PALL = pallidum, DENTG = dentate gyrus, HIPPG = hippocampal gyrus. NLP = nucleus lateralis posterior of the thalamus, NVPL = nucleus ventralis posterolateralis of the thalamus, NMD = nucleus medialis dorsalis of the thalamus, NVPM = nucleus ventralis posteromedialis of the thalamus, SUBN = substantia nigra, CC = corpus callosum, STT = spinothalamic tract, SFG = superior frontal gyrus, CING = cingulate gyrus, SMED = striae medullaris, CG = central gray.

sections in Figure 1 which are designated by A:17 L:10, A:15 L:10, and A:10 L:12 each represent an  $N$  of 2. The other sections represent  $N$ 's as follows: A:8 L:5,  $N = 3$ ; A:6 L:5,  $N = 4$ ; A:5 L:5,  $N = 1$ ; and, finally, A:2 L:2,  $N = 6$ . The three reproductions on the left of Figure 1 are at different anterior-posterior levels in the amygdala, that at the top being most anterior. The remaining four are at four different anterior-posterior levels through the pons and thalamus, that at the top right being most anterior. The anatomical structure in which each lead was found to be located is identified by familiar abbreviations. The label for the particular behavior elicited by electrical stimulation is shown prefixed to the abbreviation of the structure from which the behavior was elicited. Stimulating in the following structures elicited the CFR:

*The amygdaloid-hippocampal complex.* The complicated arrangement of nuclei within the amygdala makes it difficult to determine for certain from which of the amygdaloid nuclei the CFR was obtained. However, as illustrated in Figure 2 and in the sections on the left of Figure 1, it is probable that the CFR was elicited only from the medial amygdaloid nucleus and from tissue adjacent to this nucleus in the superior bank of the rhinal fissure. That these effects were specific to this nucleus is suggested by the fact that they were not obtained, as illustrated in A:15 L:10, 2 mm. posterior to it (probably in basal amygdaloid nucleus), nor 4 mm. superior to it (probably in central amygdaloid nucleus). Stimulating the anterior portion of the hippocampus resulted in the slow response, not the CFR.



FIG. 2. Photograph of the section in the plane of the electrode of one of the brains represented as A:17 L:10 in Figure 1.

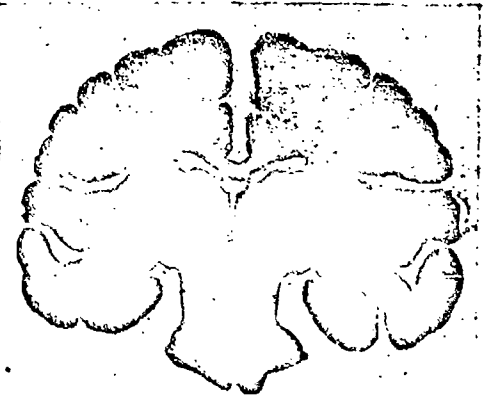


FIG. 3. Photograph of the section in the plane of the electrode of one of the brains represented in A:2 L:2 in Figure 1.

*The corpus striatum.* As illustrated in the three sections on the left of Figure 1, the CFR was elicited from the corpus striatum only when the lead was in the posterior portion of the pallidum or in the external medullary lamina surrounding the pallidum. Stimulation 2 to 4 mm. anterior to these structures in the striatum, particularly in the putamen, inhibited responses.

*Thalamus.* The only thalamic nuclei from which the CFR was elicited were, as illustrated in A:8 L:5 and A:5 L:5 of Figure 1, the ventral nuclei VPL and VPM. Motor effects were obtained from the lateral thalamic nuclei while no apparent effect was observed during stimulation in medialis dorsalis.

*Mesencephalon.* The CFR was elicited from several structures in the mesencephalon. Whenever the trigeminal nerve was involved, this response was very frantic, and the monkey screamed and rubbed its face. This was apparent from stimulating (a) where the root of the nerve appears, as in A:8 L:5 just lateral to the pons (Gasserian ganglion); (b), where it enters the pons through the superolateral aspect of the brachium pontis (not illustrated, but 1 mm. posterior and medial to the tract shown in A:8 L:5); and (c) where the mesencephalic root of the nerve runs close to periaqueductal central gray, as illustrated in Figure 3 and A:2 L:2.

Similarly, whenever stimulation in the pons may have involved the spinothalamic tract, as in A:5 L:5 and in A:2 L:2, the CFR was elicited, sometimes accompanied by screaming.



However, when, as in A:2 L:2, the lead was clearly in periaqueductal central gray, where it probably did not involve either spinothalamic tract or trigeminal nerve, the CFR was always elicited accompanied by autonomic signs but *never* by screaming or face-rubbing.

*Other structures.* The CFR was *not* elicited from sensorimotor cortex, sensorimotor pathways, n.VL and pulvinar of the thalamus, substantia nigra, or tegmentum inferior to central gray.

It is apparent in Figure 1 that the motor effects followed stimulation mainly of sensorimotor pathways and thalamic nuclei projecting to the motor cortex, while responses were inhibited by stimulating primarily in corpus striatum but also in ansa peduncularis.

#### DISCUSSION

Many investigators have elicited fearful reactions in animals by electrically stimulating in some subcortical structures. In this study a conditioned fear response invariably followed electrical stimulation in certain of these structures in the monkey. More recently, Delgado, Roberts, and Miller (7) have demonstrated that stimulation in some of these structures in cats leads to fear-like behavior which can be conditioned, can be used to motivate trial-and-error learning, and can function as punishment to make hungry animals avoid food. It may be concluded, therefore, that responses resulting from this stimulation, unlike those elicited by stimulating in the hypothalamus, are not "pseudo-affective" responses but, instead, are responses which have the characteristics of learned behavior in that they can be adapted to external circumstances.

There is a question, however, as to whether these elicited responses are actually fear responses. In the present study there are three other possible interpretations: (a) One alternative is that the stimulation was in "pain fibers" and therefore elicited a pain-escape response rather than a pain-avoidance response (CFR). This is a plausible interpretation, since overturning the left cup was the conditioned response for both conditions. Indeed, in the case of stimulation of structures related to sensory fibers, such as trigeminal nerve, spinothalamic tract, pallidum, and ventral thalamus, it is a likely interpretation.

Such is probably not the case for stimulation in amygdala and central gray, however, for these structures are not related to sensory fibers. (b) A second, less likely alternative is that stimulation was in auditory structures involved in the transmission of the conditioned fear stimulus (high tone). Two factors argue against this interpretation: First, it is unlikely that stimulation would selectively evoke responses to the high tone; second, the structures from which the fear responses were evoked are remote from those related to the auditory system. (c) Finally, it is possible that the stimulation resulted merely in a novel sensation to which the animal responded with the fear response. This interpretation is quite unlikely since the animals had learned to respond to the other (left, not the right) cup when confronted with novel external stimuli (the no-tone condition). It seems reasonable, therefore, to advance the interpretation that electrical stimulation, at least in the amygdala and central gray, induces in the animal a condition similar to that which is present when it is anxious or afraid of being hurt.

Papez (17) and more recently, MacLean (13), have summarized the evidence to support the conclusion that emotional expression is mediated through the related cerebral structures called the limbic system. It is well established that the amygdala is included in this system. Further, Nauta (15) has recently demonstrated that periaqueductal central gray is also related to the limbic system by way of collateral fibers from the fornix. These considerations perhaps provide a rationale for the findings that fearful behavior was elicited by stimulating in central gray and medial nucleus of the amygdala.

A difficulty for this formulation is that fear was not elicited by stimulating in other amygdaloid nuclei or in the anterior hippocampus, i.e., structures which are also included in the limbic system. Kaada (11), and MacLean and Delgado (12), however, have reported "fear" responses following stimulation in these structures. Some of the factors which might be responsible for these discrepant results can be pointed out. Stimulating at two different points within what appears to be the same anatomical structure may produce

different behavioral effects. Thus, in the present study, stimulation in the corpus striatum through one lead resulted in motor effects but through an adjacent lead resulted in inhibitory effects. Secondly, what appear to be minor variations in the parameters of stimulation produce different behavioral effects. Thus, stimulating in putamen with 100-cps current in the present study resulted in inhibition, but stimulating in the putamen with 60 cps in an earlier study (19) resulted in hyperactivity. The resolution of these problems would require a systematic analysis of the effect on behavior of point-by-point stimulations throughout a structure in question, varying all the parameters of the stimulating current. Until such systematic studies are undertaken, assigning functions to structures based on the results of electrical stimulation is hazardous.

At present, however, it seems profitable to include the results of the present study among those which support the notion that the limbic system is involved in the expression of emotions.

#### SUMMARY

1. An assembly containing six electrodes was implanted in mesencephalic, diencephalic, and rhinencephalic structures in the brains of 10 monkeys (*Macaca mulatta*), which had been trained to avoid shock to the feet.

2. Electrical stimulation of some structures evoked a response identical to the response to avoid shock; of other structures, an inhibitory response; of other structures, motor effects; and of some structures no apparent response.

3. Stimulation of the following structures elicited the conditioned fear response: medial nucleus of the amygdala and adjacent tissue in rhinal fissure, trigeminal nerve at the Gasserian ganglion, rostral part of the pons, medial part of the mesencephalon in the vicinity of the central gray, nucleus ventralis posteromedialis, external part of the nucleus ventralis posterolateralis of the thalamus, and external medullary lamina of the pallidum.

4. Electrical stimulation of the following structures did not evoke the avoidance response: sensorimotor cortex, sensorimotor pathways, nucleus ventralis lateralis of the thalamus, pulvinar of the thalamus, substantia nigra, part of the tegmentum inferior to

the central gray, anterior hippocampus, posterior portions of the amygdaloid nucleus, and the putamen.

5. A great variety of motor effects affecting head, eyes, face, forelimbs, hindlimbs, and also the tail were evoked by stimulation. In some instances responses were inhibited by stimulation.

6. The results suggest that fear may be induced by electrical stimulation of some structures, not others. The structures from which fear was elicited appear to be related to the limbic system.

7. These results are interpreted as indicating that electrical stimulation of some subcortical structures elicits fear responses which may be adapted to external circumstances.

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